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13. Abstract: Many studies have shown that methionine restriction inhibits growth of a variety of human tumor xenografts, including prostate cancers. In contrast, methionine restriction is relatively well tolerated by normal host tissues. The overall goal of the current project is to clarify the molecular mechanisms by which methionine restriction inhibits tumor growth. During the first year of support, we focused on Specific Aim 1, which is to determine whether methionine restriction increases oxidative stress in human prostate cancer cells. We used an established biochemical assay to measure intracellular glutathione levels and glutathione export. The assay enabled us to distinguish reduced glutathione (GSH) from glutathione disulfide (GSSG), an oxidized form. We found that methionine restriction had no appreciable affect on total intracellular glutathione content in PC-3 prostate cancer cells, or on the ratio of GSH/GSSG or glutathione export from those cells. We plan to confirm these results with additional control experiments with known inhibitors of glutathione synthesis and normal rat hepatocytes. If confirmed, these results indicate that methionine auxotrophy of tumors is probably not related to the role of methionine in glutathione synthesis and homeostasis, but rather to its role as a methyl donor, as proposed for Specific Aim 3.

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Introduction

Many animal studies have shown that dietary methionine restriction inhibits growth of a variety of human tumor xenografts, including prostate cancers. In contrast, methionine restriction is well tolerated by normal host tissues for prolonged periods. Recent cell culture studies carried out in the principal investigator's laboratory have defined some of the molecular mechanisms by which methionine restriction inhibits prostate cancer cell growth. On the basis of these preclinical observations, we **hypothesize** that dietary methionine restriction exerts a tumor specific growth inhibitory effect while having minimal deleterious effects on normal tissues.

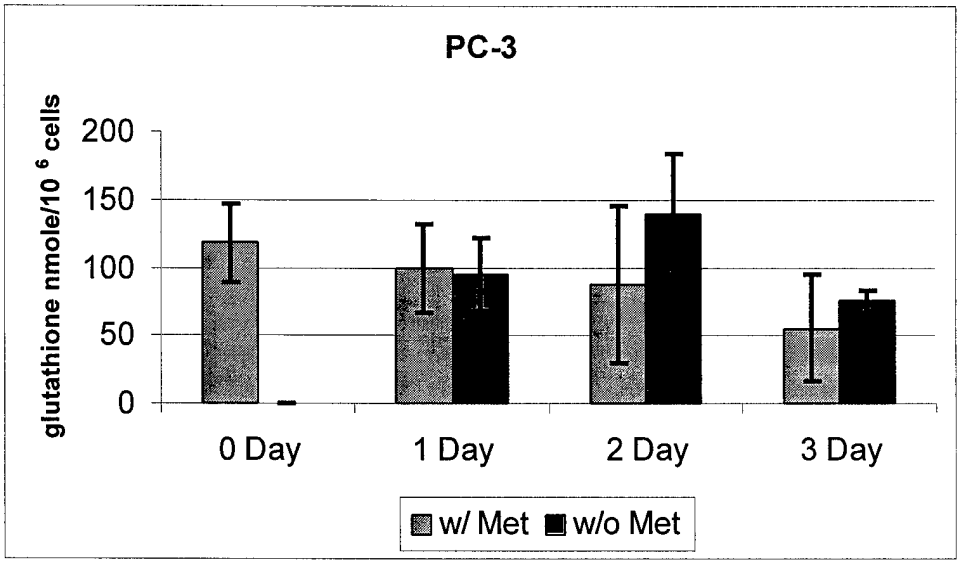
As a preliminary test of this hypothesis, we recently began a phase I clinical trial of dietary methionine restriction for adults with refractory solid tumors at Baylor College of Medicine. Twelve patients have enrolled so far. Patients in the trial received no cancer treatments other than the dietary modification. Dietary methionine restriction was well tolerated for several weeks and reduced plasma methionine levels from 21.6 ± 7.3 μM to 9 ± 4 μM within two weeks, representing a 58% decline. The levels achieved were below the concentration that suppresses tumor cell growth in vitro. In addition, two patients experienced objective responses. One elderly patient with hormone independent prostate cancer exhibited a >25% fall in prostate specific antigen concentration. Although preliminary, these results strongly suggest that dietary methionine restriction is active against hormone independent prostate cancer.

The overall goal of the current project is to clarify the molecular mechanisms by which dietary methionine restriction inhibits tumor growth. In preliminary experiments, we found that methionine restriction caused human prostate cancer cells to undergo "mitotic death", which is known to be initiated by DNA damage. Methionine restriction also led to induction of several DNA-damage-inducible genes. We therefore **hypothesize** that methionine restriction initiates cell cycle arrest and death of cancer cells by causing DNA damage.

Endogenous reactive oxygen species from mitochondria are a major cause of oxidative DNA damage. Glutathione, a ubiquitous reducing agent that is partially synthesized from methionine, functions to minimize oxidative stress. Our work so far has focused on **Specific Aim 1,** which is to determine whether methionine restriction increases oxidative stress in human prostate cancer cells.

Body

We used an established biochemical assay [1] to determine whether methionine restriction affected intracellular glutathione levels in PC-3 human prostate cancer cells or glutathione export from those cells. Cells were seeded in 6 well plates in complete medium containing 100 μM methionine + 10% FBS. One day thereafter, they were divided in two groups and re-fed either complete medium or medium containing 100 μM homocysteine in place of methionine. At time zero or one or two days later, medium was collected from wells for analysis, and cells were lysed in 1 ml ice cold 10% 5-sulfosalicylic acid. Total glutathione content was then measured from cell lysates and medium samples in a reaction mixture containing NADPH, DTNB, cell lysate, and glutathione reductase as described previously [1]. The reaction yielded 2-nitro-5-thiobenzoic acid, which was monitored at 412 nm with a spectrophotometer. Glutathione content directly correlated with rate of A_{412} increase. Before testing cell lysates or medium samples, we first developed standard curves with commercially available GSH and GSSG standards. Assays were repeated at least three times to yield results depicted below.



It is apparent from the above graph that total intracellular glutathione content did not fall in response to methionine restriction as originally hypothesized. In fact, there appeared to be an insignificant *rise* in total intracellular glutathione content two and three days after methionine restriction. Futhermore, export of glutathione into growth medium did not increase in response to methionine restriction (not shown).

We also did a series of experiments in which we added 2-vinylpyridine to the glutathione reaction mixture in order to derivatize reduced GSH and thereby measure remaining glutathione disulfide (GSSG), an oxidized form of GSH that is not derivitized by 2-vinylpyridine. We found that >90% of intracellular glutathione consisted of the reduced form (GSH), and that GSSG content was not affected by methionine restriction (not shown).

Key Research Accomplishments

During the first year of support, we focused on Specific Aim 1. In summary, our preliminary results indicate that methionine restriction had no appreciable affect on total intracellular glutathione content in PC-3 prostate cancer cells, or on the ratio of GSH/GSSG or glutathione export from those cells. We plan to confirm these results with additional control experiments (see below). If confirmed, these results are important, since they indicate that methionine auxotrophy of tumors is probably not related to the role of methionine in glutathione synthesis and homeostasis, but rather to its role as a methyl donor, as proposed for Specific Aim 3.

Reportable Outcomes (publications resulting from DOD support)

1. Lu, S, Hoestje, SM, Choo, E, **Epner, DE**. Methionine restriction induces apoptosis in prostate cancer via the c-Jun N-terminal kinase-mediated signaling pathway. 2002, Cancer Letters, 179:51.-8.
2. Lu, S, Hoestje, SM, Choo, E, **Epner, DE.** Induction of Caspase-Dependent and -Independent Apoptosis in Response to Methionine Restriction. Submitted to Biochemical and Biophysical Research Communications.